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NATURAL HISTORY AND LIFE-CYCLE STAGES OF *NOTIPHILA CARINATA* (DIPTERA: EPHYDRIDAE)

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Abstract.—Adults of *Notiphila carinata* Loew have been collected in eight states, all east of the Mississippi Valley and within the distribution range of *Justicia americana* (L.) Vahl, or water willow, the only macrophyte species with which the larvae and puparia have been associated. Adults were observed feeding on exposed periphyton on stems and leaves of water willow; females deposited eggs in irregular masses of 3–20 on folded or curled leaves on or near the water or mud surface. The incubation period for 35 eggs ranged from 1 to 3 days at 18–24°C. The first larval stadium was not precisely measured, but it was definitely more than 3 days in laboratory cultures. The second stadium was not measured, but the third ranged from 42 to 44 days for 3 overwintering specimens and the puparial phase ranged from 12 to 18 days for 3 specimens.

Larvae are metapneustic in all instars. The posterior spiracles are retractile, aciculate, and contiguous, with the spiracular atria often fused. The larva forces its needle-like spiracles into the aerenchyma of water-willow roots from which it apparently procures oxygen. However, available evidence indicates that third instars, and perhaps earlier instars, move about for long periods of time (up to 8 days in laboratory) between insertions of spiracles in the roots. Larvae of all instars ingested sapropel, or black ooze, which surrounded the roots of many water-willow plants. The high transparency of the larval integument greatly facilitated larval feeding observations. The species overwintered in Ohio only as late second- or third-instar larvae between the clustered roots of water willow.

All stages of the life cycle are described and illustrated.

In North America the 69 genera and 377 species of Ephydridae presently recorded make them the largest drosophiloid family on the continent. However, this large family of semiaquatic and aquatic acalyptrate flies has been so little studied biologically that the life histories of only relatvely few species have been described.

Nearly all of the known larvae and puparia of Ephydridae are aquatic—living and feeding in algal mats, diatom-covered mud flats, wet sand shores, and black ooze, or sapropel (Brock *et al.*, 1969; Dahl, 1959; Deonier, 1961, 1974; Deonier and Regensburg, 1978; Eastin and Foote, 1971; and others). The larvae of the notiphiline genus *Hydrellia* live and feed in the leaves and stems of aquatic macrophytes (Deonier, 1971) and those of various other genera scavenge on dead noninsectan animals (Bohart and Gressitt, 1951;

Disney, 1969; Soan and Adolph, 1971; Wirth, 1971; and others), parasitize spider eggs and frog eggs (Becker, 1926; Bokerman, 1957), or scavenge on dead oil-pool-entrapped insects (Crawford, 1912; Thorpe, 1930).

For the genus *Notiphila*, Müller (1922) and Torelli (1922) presented the first known descriptions of any immature stages. Müller gave a brief description of the larva of *N. riparia* Meigen and Torelli described and figured the immature stages of *N. chamaeleon* Becker. Varley (1937), in a paper on root-piercing aquatic insect larvae, discussed the respiratory modifications in the puparium of *N. riparia*. Meijere (1941) recorded *Notiphila brunnipes* Robineau-Desvoidy as mining the roots of *Nymphaea*. Houlihan (1969) described at length the spiracular structure and root-piercing behavior of *N. riparia* and presented valuable data on the use of roots of various plant species by the larvae in relation to epidermal thicknesses or distances to root gas spaces.

Until very recently, the only life-history information available on Nearctic *Notiphila* was that presented by Berg (1950) for *N. loewi* Cresson. However, Busacca and Foote (1978) have now contributed significantly to the knowledge base for Nearctic *Notiphila* with their work on the life histories and immature stages of *N. aenigma* Cresson and *N. solita* Walker.

Materials and Methods

Larvae and puparia were collected by sieving, with a U.S. Standard Sieve Series, substrates from water-willow root systems previously extracted by spade. Eggs were obtained by caging gravid females. Larvae and puparia were reared both in individual culture bowls containing sapropel and one water-willow plant and en masse in a transplanted culture of water willows. The pH was measured with a Hach portable pH meter.

All larvae and puparia were collected in Four-Mile Creek near Oxford, Butler Co., Ohio, and in Brashear's Creek near the Salt River, Spencer Co., Kentucky. Field observations were made principally at the Four-Mile Creek site and secondarily at Adams Lake, Adams Co., Ohio.

Voucher specimens for the life history and immature stages are in the Water Resources Laboratory of the University of Louisville and the collection of D. L. Deonier. Adult specimens studied are principally in the National Museum of Natural History (Smithsonian Institution) and the Miami University Insect Collection.

Abbreviations

AN, anus; ANT, antenna; ARR, aristal ray; BP, basal part of mouthhook; DB, dorsal bridge; DC, dorsal cornu; DCC, dorsocephalic cap; DT, dorsal tibial macrochaeta; ES, epistomal sclerite; FA, facial macrochaeta; FC,

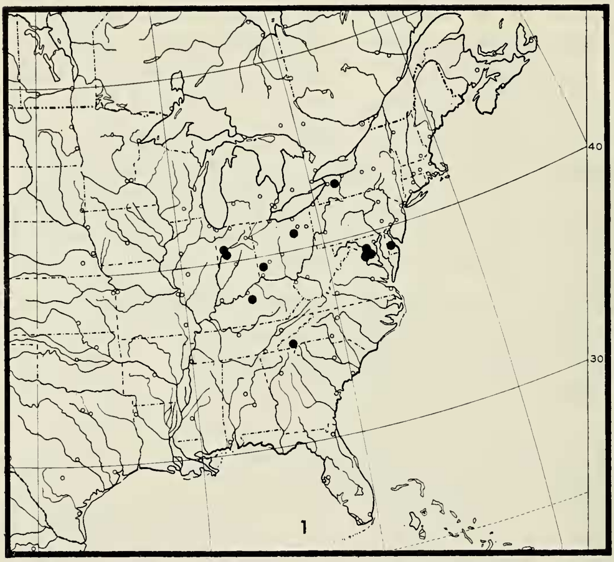


Fig. 1. *Notiphila carinata.* Distribution of collection localities of adults.

femoral comb; FS, furcate structure; GE, genal macrochaeta; HB, hypostomal bridge; HL, head-lobe; HP, hook part of mouthhook; HS, hypostomal sclerite; $LPT_{1,2,3}$, lateral postanal tubercles; LS, ligulate sclerite; MH, mouthhook; MP, micropyle; PAE, postanal elongation; PAP, perianal pad; PB, parastomal bar; PSP, posterior spiracle; SA, spiracular atrium; SSP, subantennal sensory plate; TH_1, prothorax; VC, ventral cornu; VPT, ventral postanal tubercle.

Natural History

Adult.—The adults of *Notiphila carinata,* some of the smallest in the genus, have been collected in eight states in the eastern United States (Fig. 1). This distribution is entirely within the distributional range of the only known larval host plant, *Justicia* (=*Dianthera*) *americana* (L.) Vahl, which occurs in North America south of Quebec and east from the Mississippi Valley. Adults have been collected from marsh-reeds, sedge-meadow, grass-shore, limnic-wrack, and floating-vegetation types of habitat, but the single most consistent habitat of many adults and all larvae has been *Justicia,* or water willow.

Adults of *N. carinata* are not common. Observed adult populations were only moderately abundant and fewer than 150 preserved specimens were available from collections of North American museums. The paucity of adults, however, may be an artifact of our observational and collecting techniques. Of the preserved specimens studied, over half were from one site, Plummers Island, Maryland, and were the recovered prey from a number of completely and partially provisioned cells of the Hibiscus Wasp, *Ectemnius* (*Hypocrabro*) *paucimaculatus* (Packard) (Krombein, 1964).

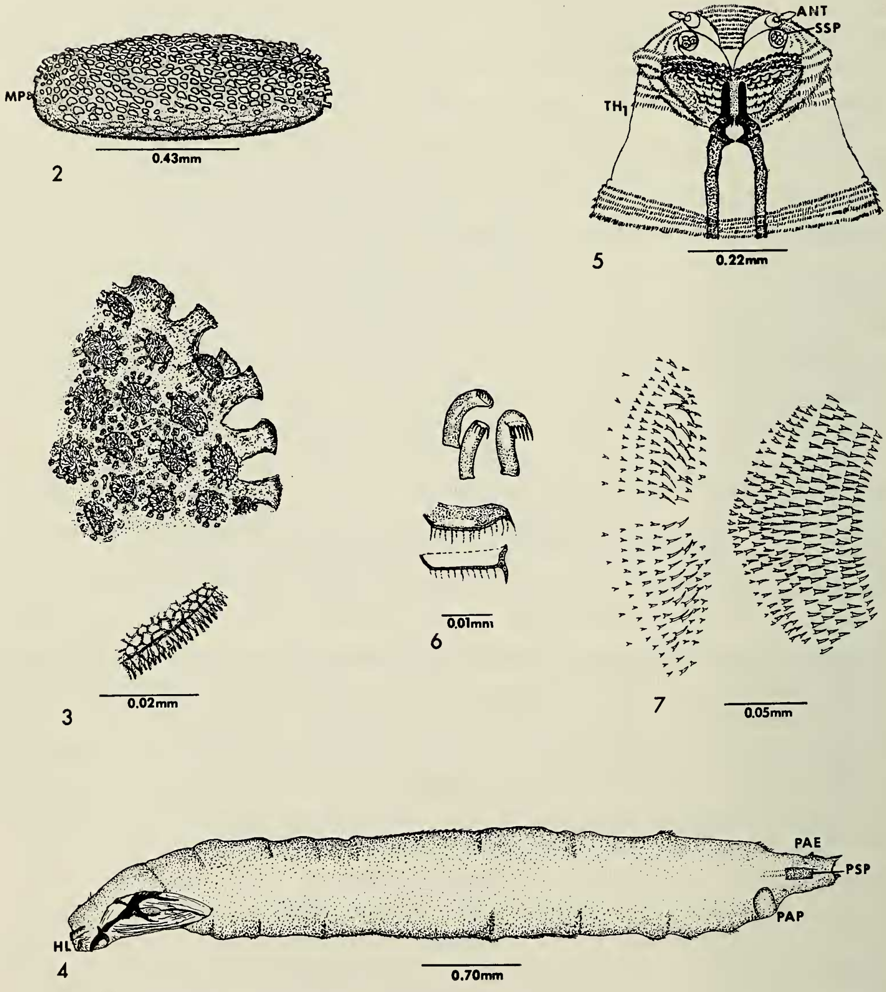
The gut contents of three specimens and scrapings from the labella of three dry-mounted specimens as well as close observations of feeding in laboratory and field indicate that the adults feed on periphyton. The gut contents appeared to contain green and yellow pigments and the labellar scrapings consisted of probable fungal fragments, sand particles, and naviculoid diatoms. Busacca and Foote (1978) observed adults of *N. aenigma* and *N. solita* applying their mouthparts to surfaces of emergent vegetation and surmised that they probably graze on microflora on the emergent vegetation. When abducted or spread, the labella form a wide, shallow inverted cup lined with the cross-striated canaliculi and having a flexible margin.

As with several congeners, adults of this species seemed to spend much of the daytime resting head downward on the lower branches of water willow, although we did frequently observe them walking and feeding over leaves on the water or mud surface. Courtship and copulation were not observed. Oviposition occurred on leaves, often curled or folded ones, on or near the water or mud surface. The females moved slowly and intermittently over their ovisites while they laid irregular masses of 3 to 20 eggs. In the laboratory, newly laid eggs could be moved, but day-old eggs were mostly cemented to the ovisite. One caged female, when not provided with water-willow leaves, oviposited on sphagnum moss and glass-cage walls.

Although we were unable to measure maximum egg capacity, we did obtain some production data. One of 2 females collected September 13, 1977 laid 35 eggs in a 24-hour period and the other laid 27 eggs in a 72-hour period. Also, a gravid female collected October 6, 1977 was found to have ovaries consisting of 6 ovarioles each and containing a total of 22 recognizable oocytes, including 12 moderately late-stage and 10 without well-differentiated choria. Since the mean daily temperature was decreasing and adults were scarce by this collection date, these data likely include some seasonal influence. Busacca and Foote (1978) estimated the total egg production for *N. aenigma* to be 110 to 180.

Egg.—The incubation period ranged from 1–3 days for 35 eggs at 18–24°C.

Newly laid eggs are pure white and, in contrast to the illustrated eggs of other *Notiphila* (*chamaeleon* by Torelli, 1922; *annulipes* by Dahl, 1959; and *aenigma* and *solita* by Busacca and Foote, 1978), the upper half of the chorion is deeply sculptured into numerous erect and semierect projections (Fig. 2). Since the overall appearance of this sculpturing is one of an extensive aeropyle, or respiratory plastron surface, it may be an adaptation to the more frequent inundation occurring in the labile stream and back-



Figs. 2–7. *N. carinata.* 2, egg, dorsolateral view. 3, egg chorion, upper and lower halves magnified. 4, third-instar larva, habitus, lateral view. 5, third-instar larva, head-lobe and prothorax, ventral view. 6, third-instar larva, facial-mask structures magnified. 7, third-instar larva, creeping welt and succeeding spinular zone on abdominal segment 7, ventral view.

water habitats characteristic of water willow. It could also be an egg-predator or parasite defense. The fine chorionic reticulation described for the egg of *N. aenigma* (Busacca and Foote, 1978) may serve as an aeropyle, but the relatively more stable marsh-reeds habitat of this species and the apparently higher ovisite are probably less demanding for this function.

In *N. chamaeleon*, on the other hand, the illustrations and description of the chorion and ovisite by Torelli (1922) indicate that it has a moderately developed aeropyle. The egg chorion of *N. annulipes* is apparently smooth and, according to Dahl (1959), equipped with a sharp structure on one end which allows it to be drilled into plant tissue.

Larva.—Although the necessity for keeping the larvae in ooze essentially precluded accurate measurement of the larval stadia, we were able to obtain some estimated durations for this species. The first larval stadium based on observations of 23 specimens is probably 3–5+ days. The third stadium lasted 42–44 days in 3 overwintering specimens which were collected as second-instar larvae January 4, 1978. We were unable to rear larvae entirely through the first and second stadia.

The larvae eclose from the egg by slitting one end of the chorion with their mouthhooks. Larvae are metapneustic in all instars; they possess a pair of retractile, spinous posterior spiracles which are used to pierce the roots and rootlets of water willow. We sieved larvae only from sapropel, or black ooze, around the root systems of water willow. One hundred plants without abundant sapropel around at least part of the root system yielded no larvae or puparia whereas 60 plants growing in sapropel yielded 34 second- and third-instar larvae and 10 puparia.

All field-collected larvae had sapropel in their guts. In microscopic observations of three first-instar larvae held in petri dishes containing small, isolated areas of sapropel in distilled water, the larvae showed a strong tendency to move into and remain in the sapropel cloud. Negative response to light was undoubtedly involved here, but the larvae protracted and retracted their head-lobes much faster in the sapropel indicating that active ingestion occurred there. The gut contents of a first-instar and a third-instar larva consisted of some yellow-green pigment, bacteria, sand particles, and organic detritus particles. Busacca and Foote (1978) found only bacteria and detritus in the guts of third-instar larvae of *N. aenigma* and *solita*.

Since larvae of *N. carinata* and these two species together with those of *Dichaeta caudata* (Fallén) (illustrated by Eastin and Foote, 1971) have comblike components in the facial mask, it seems probable that all are filter-feeders and that particles from the ooze are collected by the comblike structures and then transferred into the mouth during partial head-lobe involution and retraction. The mouthhooks, although possibly involved here in an ancillary fashion, retain their primary locomotory function.

Although we failed to observe actual penetration of the posterior spiracles into roots, we did observe microscopically what we interpreted as attempted penetration in which the larva was coiled around the rootlet and probing at its surface with the posterior spiracles. As Houlihan (1969) explained for *N. riparia*, the larvae protract and insert their spiracles solely by increasing their blood pressure and they therefore require the compression of mud or ooze for effective spiracular insertion. However, in the first-instar larvae of *N. carinata*, the well-developed creeping welts, which are only weakly developed in the last two larval instars, may provide anchorage needed for spiracular insertion by these small larvae as well as secure locomotion necessary for remaining on the root system.

The larvae are at least partially dependent upon the roots for oxygen, for although two third-instar larvae survived 6 and 8 days respectively in ooze without access to roots and several survived 24 hours in distilled water, we successfully reared only larvae having access to roots. Our data and those of Busacca and Foote (1978) do strongly indicate however that larvae roam free of roots for fairly long periods of time. The latter authors reported that older third-instar larvae of *N. aenigma* survived low oxygen conditions for more than a month in the laboratory and also that almost all larvae were free of *Typha* rootlets when originally collected.

We found larvae of *N. carinata* only in stands of water willow. Thorough examination of 25 plants of *Eleocharis* sp. and ten plants of *Leersia* yielded no larvae or puparia whereas 50 nearby water-willow plants yielded five puparia. Busacca and Foote (1978) discovered a similar degree of apparent specificity in *N. aenigma* and *N. solita*.

Notiphila carinata overwinters as second- and third-instar larvae in Ohio. On January 4, 1978, we collected seven second-instar and seven third-instar larvae from the sapropel around root systems of an old stand of *Justicia* covered by Four Mile Creek. We sieved ten of these from one root system. The sapropel pH ranged from 5.6–6.4. This sieving also yielded numerous larvae of four species of Tipulidae, ten species of Chironomidae, two of Tabanidae, six of Trichoptera, and one species of Elmidae. Adults of *N. carinata* became scarce in September and disappeared in October.

In order to determine something about larval dispersal in this species, we placed seven of these overwintering larvae at one point in a 120 × 55 cm tank containing a dense, uniform culture of transplanted water willow, the roots of which were free of *N. carinata* stages. After 30 days, one had moved approximately 35 cm, three 30 cm, and three undetermined distances. All of these larvae pupariated in February and the adults emerged in March.

Pupariation.—The time from pupariation to emergence of adult was 12–18 days in three specimens and approximately 18 days in two others.

When ready to pupariate the third-instar larva inserts its spiracles in a root where they remain throughout pupariation and subsequent pupation (Fig. 20). One specimen from a group of seven third-instar larvae collected January 4, 1978 required 24 hours to pupariate. During this process the posterior spiracles became thicker and darker and their slitlike spiracular orifices enlarged. The spiracles also came to be directed ventrad and often anteroventrad giving them a hooked, or acanthous shape. We found only the distal one-fifth or less of abdominal segment 8 inserted and slightly enlarged, but in larvae of *N. riparia* most of the segment must be embedded in the root before pupariation begins (Houlihan, 1969). In nature, the spiracles apparently must remain intact and inserted for successful pupation and emergence. Puparia, even some enclosing nearly pharate adults, which we accidentally detached never produced adults. Berg (1950) reported similar results with *N. loewi* and Busacca and Foote (1978) found no viable detached puparia. For *N. riparia*, Houlihan (1969) stated that "the development of the pupa and the pharate adult inside the puparium is dependent on the oxygen in the gas spaces in the plant." He found that intact, detached puparia filled with water and sank in a few days.

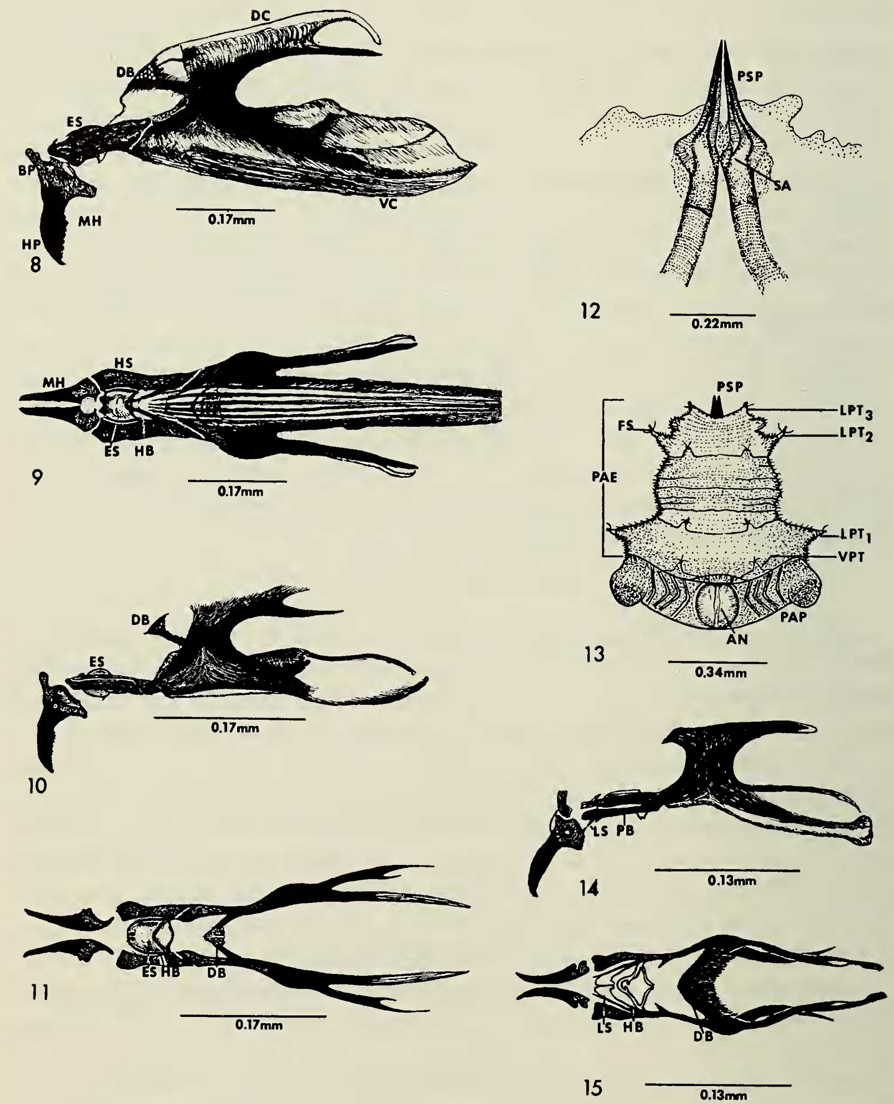
Although it was not mentioned by Houlihan (1969) or Busacca and Foote (1978), in *N. carinata* we found all puparia to have membranous, flexible postanal elongations which allowed movement of the main body through an arc of about 180°. We surmise that this is an adaptation which reduces the risk of the puparium being broken off by shifting roots and substrate and also allows greater maneuvering by the emerging adult.

Description of Adult

Diagnosis.—*Notiphila carinata* is one of the smaller members of the tribe Notiphilini and specimens may be distinguished from those of similar taxa by the following combination of character states: Length 2.58–3.57 mm; costal vein short, extending to vein R_{4+5}; 2 pairs of dorsocentral setae (1+): 1 pair of small, proclinate, fronto-orbital setae laterad of larger reclinate, fronto-orbital seta; middle leg of male with femoral comb of closely-set setae along posteroventral margin; middle leg with 3 dorsal tibial setae; antenna partially pale; mesanepisternum often with dark-colored spot; mesonotum generally immaculate; male terminalia diagnostic with widely U-shaped, ventral process of epandrium; extending arms of ventral process not curved or sinuate; and basiphallus short with slightly expanded apex.

Description.—Moderately-small to medium-sized shore flies with grayish-brown to light brown background coloration and a few darker brown markings.

Head (Fig. 18).—Head width-to-height ratio averaging 1:0.78; postfrons (frons) width-to-length ratio averaging 1:0.64; most of frons light brown, pollinose, median triangular area and lateral fronto-orbits lighter in color, generally concolorous. Dorsalmost paravertical seta large, approximtely double size of ocellars; at most with one proclinate fronto-orbital seta. Antennal segments mostly dark brown, third segment with basal half, especially posteroventral portion pale, usually yellowish; arista with 8 or 9 dorsal rays. Face lightly pollinose, unicolorous, light yellow; prefrons



Figs. 8–15. *N. carinata.* 8, third-instar larva, cephalopharyngeal skeleton, lateral view. 9, same, ventral view. 10, second-instar larva, cephalopharyngeal skeleton, lateral view. 11, same, ventral view. 12, third-instar larva, posterior spiracles, dissected, dorsal view. 13, third-instar larva, perianal pad and postanal elongation, ventral view. 14, first-instar larva, cephalopharyngeal skeleton, lateral view. 15, same, ventral view.

(face) height-to-width ratio averaging 1:0.69; facial setae small, hairlike. Eye height-to-width ratio averaging 1:0.83; eye-to-cheek ratio averaging 1:0.16. Gena narrow, generally concolorous with face, especially anteriorly, becoming grayer posteriorly; genal seta approximately subequal to dorsalmost paravertical seta. Maxillary palp pale, yellowish orange.

Thorax.—Mesonotum light brown, darker than pleural areas and generally immaculate. Mesanepisternum often with dark brown spot, especially in specimens from Atlantic Coast area. Chaetotaxy of thorax as follows (macrosetae): 2 pairs of dorsocentrals (1+1); 1 pair of prescutellar acrostichals, widely separated, situated between alignment of dorsocentrals and acrostichals; 1 pair of presuturals; 1 pair of interalars, transversely aligned with prescutellar acrostichals; 1 pair of supra-alars; 1 pair of postalars; 2 pairs of lateral scutellars, posterior pair inserted apically; 1 pair of humerals; 2 pairs of notopleurals, one inserted near each ventral corner, anterior seta larger; 2 pairs of mesanepisternals near posterior margin, dorsal one about ½ length of posterior seta; 1 pair of mesokatepisternals. Femora gray, with some darker areas along dorsum; tibiae generally concolorous with femora, although front pair slightly darker, usually with darker preapical spot or annulation; tarsi pale, yellow; setal fascicle of hind basitarsus dark. Middle leg (Fig. 19) with 3 dorsal tibial setae; middle leg of male (Fig. 19) with femoral comb of closely-set setae along posteroventral margin. Wing length-to-width ratio averaging 1:0.45; costal vein extending to vein R_{4+5}; costal vein index averaging 1:0.55; M_{1+2} index averaging 1:0.97.

Abdomen.—Abdomen length-to-width ratio of males averaging 1:0.84; length of fourth tergum to fifth tergum ratio of males averaging 1:1.1; fifth tergum width-to-length ratio of males averaging 1:0.48. Maculation pattern variable, best developed on terga 3 and 4; usually with 2 oblong spots on either side of median, sometimes with darker area connecting spots anteriorly. Male terminalia as in diagnosis.

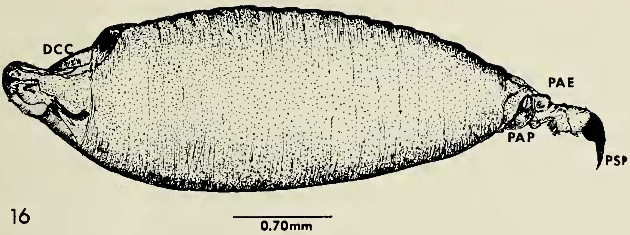
Description of Immature Stages

Egg (Figs. 2, 3).—Length 0.72–0.95 mm ($\bar{x}$ = 0.87); maximum width 0.23–0.28 mm ($\bar{x}$ = 0.87). Newly laid egg pure white, elliptical, slightly flattened ventrally, transversely convex dorsally; micropyle stalk, minute, inconspicuous; micropylar end slightly blunter than opposite end. Upper ½ of chorion deeply sculptured into 2 types of numerous erect, distally concave projections: longer, narrower ones mostly restricted to ends of egg and shorter, broader projections; lower ½ of chorion appearing finely reticulate at 90× magnification, micropapillate at 430×. (Based on 64 specimens from field-collected females.)

First-instar larva.—Length 1.17–1.70 mm; maximum width 0.23–0.28 mm. Body grayish white (integument semitransparent). Similar to third instar except in following characters: Setulosity darker, more distinct, relatively longer and setuloid. Facial mask with tips of all spinular units light to medium brown; labium padlike; prothorax with about 14 bands of spinules, all uniordinal except posterior band with longer spinules; pair of medium brown spinule patches anterolateral to mouth; meso- and metathorax each with zone of about 9–14 bands, all essentially uniordinal except about last 2 posterior bands with much longer spinules. Abdominal segments 1–7 each with distinct creeping welt composed of 2 bands of hooked microspinules directed anteriad and 2 posteriad, microspinules in 2 inner bands about twice as large as in 2 outer bands; abdominal segment 1 with 2 spinule zones each having 4–7 partially overlapping bands of posteriorly-directed, multiordinal spinules; segments 2–7 each with similar spinulosity arranged in 3 zones; perianal pad nearly rectangular, often darker with numerous small spots. Postanal elongation relatively shorter, only about 1⁄12 as long as preanal length; postanal tubercles less distinct; ventral postanal spinule patch relatively smaller; posterior spiracles very light yellow-brown, 3.2–3.8 times as long as greatest diameter and with spiracular atria fused; distal 2⁄3 of spiracle with indistinct microcribiform slits.

Cephalopharyngeal skeleton (Figs. 14, 15) length (excluding mouthhooks) 0.28–0.46 mm. Pharyngeal sclerite slightly paler; mouthhooks with ventral edge of hook part nearly straight (4–5 minute teeth visible at 430×); mouthhook part with small irregular fenestra; base part anticulated anteriorly with small, curved sclerite and dorsally with larger projecting sclerite; hook part about 1.5 times as long as greatest dimension of base part. Hypostomal and pharyngeal sclerites apparently fused; parastomal bars distinguishable parallel to and fused to hypostomal sclerite posteriorly; ligulate sclerite projecting anteriad between anterior arms of hypostomal sclerite; apparent straplike hypostomal bridge present at midlength and projecting anteroventrad behind ligulate sclerite. Dorsal bridge in lateral view, not greatly projecting, continuous with dorsal cornua; bridge, in ventral view, arching anteriad (U- or V-shaped) with wide fibrous posterior fringe. Dorsal cornua unforked distally, with slightly acute tip; ventral cornua forked into thin straplike dorsal arm and downcurving ventral arm 2–4 times as wide as and nearly meeting the dorsal arm posterodorsally; ventral cornua 1.5–1.7 times as long as dorsal cornua. Ventral length of pharyngeal sclerite 2.4–2.8 times hypostomal length; ventral cornu length 1.5–1.7 times hypostomal length. (Based on 27 reared specimens.)

Second-instar larva.—Length 3.40–3.69 mm; maximum width 0.36–0.86 mm. Body grayish white, integument including spinulosity mostly transparent. Similar to third instar except in following characters: Facial mask relatively wider, apparently with more rows of pectinate cultriform structures; prothoracic spinule bands with larger, slightly curved spinules postero-



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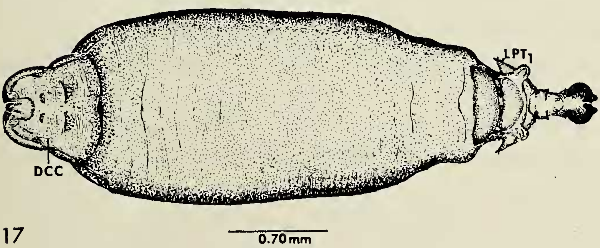

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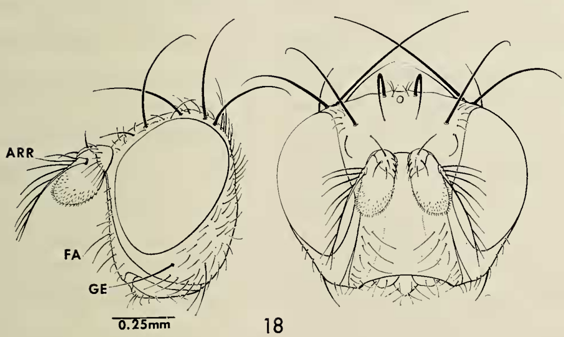

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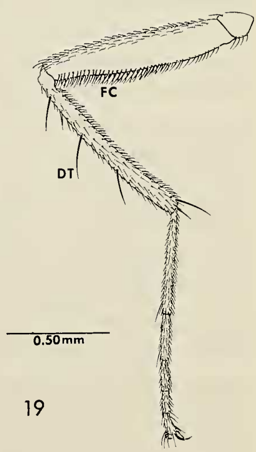

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Figs. 16–19. *N. carinata.* 16, puparium, habitus, lateral view. 17, same, dorsal view. 18, adult, head, lateral and anterior views. 19, adult, middle leg, male, posterior view.

lateral to mouth. Abdominal creeping welts somewhat fainter; lateral and dorsal spinulosity relatively slightly longer; ventral postanal spinule patch relatively smaller; postanal tubercles relatively less distinct.

Cephalopharyngeal skeleton (Figs. 10, 11) length (excluding mouthhooks)

0.42–0.53 mm. Pharyngeal sclerite dark brown or black, with hyaline periphery reduced; other sclerites light to medium brown. Mouthhooks slightly longer than hypostomal sclerite, base part darker and with distinct, small, circular fenestra; mouthhooks with 10–12 fine teeth on distal ½, 5 largest near midlength. Epistomal sclerite (plate) pale and domelike above hypostomal sclerite in lateral view with anterior end rounded, bearing small paired oval structures, and projecting between anterior hypostomal arms; apparent hypostomal bridge projecting anteroventrad in V-shape below epistomal sclerite. Pharyngeal sclerite with dorsal bridge straplike, smaller and sharply V-shaped in ventral view; dorsal cornua ending in open V-shaped recess with lower arm 2–3 times as long as upper arm of recess; posterior ½ of ventral cornua hyaline with dark border usually incomplete at posterior end; dark part of ventral cornua V-shaped in lateral view; ventral cornua 1.5–1.8 times as long as dorsal cornua. Ventral length of pharyngeal sclerite 3.4–3.8 times hypostomal length; ventral cornu length 2.5–2.8 times hypostomal length. (Based on 3 field-collected specimens.)

Third-instar larva (Figs. 4–7, 12, 13).—Length 3.06–6.75 mm; maximum width 0.54–1.17 mm. White or creamy white except very light-brown posterior spiracles; integument including nearly all spinulosity transparent; body subcylindrical, tapering only very slightly from middle to head-lobe and to perianal pad. Head-lobe (segment 1 of authors) retractile, bilobate (Fig. 5) with each lobe bearing 3-segmented antenna distally and subantennal sensory plate ventrally; antennae slightly longer than mouthhooks, segment 3 elliptical, about 3 times as long as wide, and slightly longer than segment 2; pair of subantennal sensory plates round and about same diameter as antennal segment 1. Facial mask (Figs. 5, 6) with 3 rows of decumbent, palmately-tipped spinules anterior to mouth, 1 row of pectinate cultriform structures lateral to mouth, and 4 rows of apically hooked, pectinate plates posterolateral to mouth; labium indistinct. Prothorax (segment 2 of authors) with 14–16 bands of minute microspinules on anterior ⅓–½, posterior ½ apparently glabrous; tracheae for anterior spiracles visible but spiracles absent; meso- and metathorax each with 6–8 bands of microspinules and 8 bands of slightly larger spinules on anterior ½–⅔ followed by glabrous posterior zone. Abdominal segments 1–7 each with numerous bands of spinules, indistinct glabrous zone, slight dorsal protuberance, and slight creeping welt distinguishable by 4–9 close-set rows having longer, posteromedially curved ventrolateral spinules (Fig. 7); anterior welt preceded by 2 zones of about 14 bands of microspinules separated by a narrow glabrous zone; remaining welts preceded by zone of 9–14 bands of short, slightly hooked microspinules and zone of straight slightly larger spinules arranged in multiordinate bands and increasing from about 6 on anterior to 14 on posterior segments; bands of slightly hooked microspinules more widely spaced ventromedially; posterior creeping welt separated from perianal pad by wide zone of 6–9 bands of multiordinal straight spinules. Perianal pad convex anteromedially, extending laterally onto sides of segment as roundly bulging lobes; 14–22 close-set, usually pigmented spinules in ventral-postanal patch between inconspicuous, ventral postanal tubercles. Postanal elongation (respiratory or breathing tube of authors) of abdominal segment 8 about 1⁄10 as long as preanal length, tapering very slightly to posterior spiracles, with 3 pairs of lateral, 1 ventral pair just posterior to perianal pad, and 1–2 pairs of somewhat irregular dorsal and ventral postanal tubercles (first lateral pair posterolateral to perianal pad); tubercles with terminal furcate structure (rayed sensilla of authors); postanal elongation partly retractile, with numerous annulations and about 30 bands of setuloid spinules. Posterior spiracles completely retractile, light brown, straight aciculate, contiguous or nearly so throughout, and often with spiracular atria fused; spiracles 2.6–3.3 times as long as greatest diameter and only about 2⁄3 as long as postanal elongation; distal 1⁄2–2⁄3 of spiracle with spiracular orifices inconspicuous dorsal, ventral, and lateral microcribiform slits.

Cephalopharyngeal skeleton (Figs. 8, 9) length (excluding mouthhooks) 0.42–0.67 mm. Pharyngeal sclerite dark brown or black centrally, with wide fiber-streaked hyaline periphery; other sclerites generally light to medium brown. Mouthhooks paired, separate basally and slightly shorter than hypostomal sclerite; hook part black, slightly or not at all decurved, with 6–8 teeth (largest pyriform and at midlength); base part medium brown, faintly fenestrate, and with conspicuous dorsal and ventral projections; base part articulating posteriorly with anterior arm of hypostomal sclerite; hook part slightly shorter than greatest dimension of base part (including dorsal and ventral projections). Hypostomal sclerite, in ventral view, broad, with ends of posterior arms acutely angled and juxtaposed to ends of pharyngeal sclerite and with very narrow hypostomal bridge projecting anteroventrad to point below posterior margin of light-brown, shield-shaped epistomal sclerite (plate); anterior margin of epistomal sclerite with paired minute medial toothlike projections (= ligulate sclerite?). Hypostomal and pharyngeal sclerites not fused; dorsal bridge, in ventral view, strongly arched anteriad and, in lateral view, steeply angled (about 45°); bridge multifenestrate and reticulate posteromedially; dorsal cornua ending in deep, often nearly closed obovate recess, with lower arm sometimes projecting beyond upper arm; ventral cornua 1.7–2.8 times as long as dorsal cornua, widest at midlength (1.5–2.0 times dorsal cornu width) and with undulant, mainly hyaline posterodorsal margin; 7–9 ventral pharyngeal (cibarial) grooves visible in ventral view. Ventral length of pharyngeal sclerite 3.4–4.2 times hypostomal length; ventral cornu length 2.2–3.0 times hypostomal length. (Based on 6 reared and 15 field-collected specimens.)

Fig. 20. *N. carinata.* 20, puparia attached to roots of water willow, *in situ.*

Puparium (Figs. 16, 17).—Length 3.50–4.95 mm ($\bar{x}$ = 4.24); maximum width 0.99–1.80 mm ($\bar{x}$ = 1.33). Empty puparium translucent, light to medium brown, fusiform with dorsal surface slightly flatter than ventral surface. Anterior spiracles absent; dorsocephalic cap rounded anterolaterally, indented anteromedially at longitudinally rugulose head-lobe scar, and undulant posteriorly; cap, in dorsal view, often with large and small pair of crescentric depressions and showing 3 zones of numerous fine, close-set transverse ridges bearing extremely minute longitudinal interconnecting fibrils, with apparent microspinules in 2 rows on metathorax. Puparium with 18 annuli between dorsocephalic cap and perianal pad, each with numerous faint, close-set, transverse micro-ridges and 6–14 bands of transparent microspinules; microspinules slightly larger on abdominal segments 3–7; abdominal venter with bands expanded into 7 indistinct creeping-welt patterns; segment 8 (above perianal pad) with 2 flat, dorsal, platelike areas and 6–8 bands of longer spinules immediately posterior to perianal pad. Perianal pad with anteromedial margin often arched anteriad between roundly bulging lateral lobes (extending onto sides of segment); pad with sclerotized chevron-shaped ridges in membrane; ventral postanal spinule patch present between relatively small ventral postanal tubercles. Postanal elongation paler, largely membranous (flexible), annulated, and about 1/10 as long as preanal length; postanal elongation with 3 pairs of lateral postanal tubercles—a large pair slightly posterolateral to perianal pad and smaller pairs near and at posterior end—and 2–3 pairs of somewhat irregular dorsal and ventral tubercles; tubercles mostly with terminal furcate structure. Posterior spiracles aciculate, black, thicker than in third instar, directed ventrad and often acanthous (curving anteriad); spiracles 2.4–2.8 times as long as greatest diameter and about as long as postanal elongation; distal 1/2–2/3 of spiracle twisted, with dorsal, ventral, and lateral micro-cribiform slitlike spiracular orifices. (Based on 5 reared and 30 field-collected specimens.)

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